Membrane Molecular Aspects of Tubule Solute Transport

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During recent years studies with isolated plasma membranes in general and transport experiments with plasma-membrane vesicles in particular (Kinne, 1976) have provided important information on the transport systems and the driving forces involved in the solute transport of the renal proximal tubule. The results of these studies are compiled in Fig. 1. In principle two driving forces can be distinguished, ATP hydrolysis for primary active transport processes and the Na\(^+\) gradient for secondary active-transport processes; the corresponding transport systems are ion-dependent ATPases and Na\(^+\)-co-transport systems. Na/K-ATPase and Ca-ATPase are present only in the basal-lateral (contraluminal) plasma membranes of the proximal tubular epithelial cells, the luminal membrane is supposed to contain a HCO\(_3^-\) stimulatable Mg-ATPase. Na/K-ATPase is involved in the active extrusion of Na\(^+\) out of the cell, Ca-ATPase activity seems to be related to an ATP-dependent movement of Ca\(^{2+}\) across the contraluminal cell border and the HCO\(_3^-\) stimulatable Mg-ATPase is thought to represent an ATP-driven proton pump. Na\(^+\)-co-transport systems are also distributed asymmetrically within the cellular plasma-membrane envelope: d-glucose, phosphate, L-lactate, neutral and basic amino acids are transported across the brush border membrane by Na\(^+\)-co-transport systems, whereas their exit from the cell involves Na\(^+\) independent transport systems. Acidic amino acids on the other hand are taken up into the cell both from the luminal and the contraluminal side by Na\(^+\)-dependent systems. For protons and Ca\(^{2+}\) there exist, in addition to ATPases, Na\(^+\)-exchange systems; a proton-Na\(^+\) exchange system is localized in the brush-border membranes and a Ca\(^{2+}\)/Na\(^+\) exchange system is localized in the basal-lateral plasma membranes.

For the studies mentioned above it is important that the membranes (including their transport machinery) be left essentially unaltered by the purification procedures, so that the isolated sheets or vesicles reflect very closely the functions of the plasma membrane of the intact cell in vivo. This goal places this area of investigation at the border line between the classical physiological approach, which wants to understand organ function

* Abbreviations: Na/K-, Ca- and Mg-ATPases; Na\(^+\)/K\(^+\)-dependent, Ca\(^{2+}\)-dependent and Mg\(^{2+}\)-dependent adenosine triphosphatase.

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**Fig. 1.** Distribution of Na\(^+\)-co-transport systems and ATP-dependent ion-transport systems within the plasma-membrane envelope of proximal-tubule epithelial cells

For further explanation see text and Kinne (1976).
Parathyrin is thought to inhibit via cyclic AMP the luminal phosphate-Na\textsuperscript{+} co-transport systems. During phosphate depletion (low \(P_f\) diet) the transport capacity of the tubule is increased probably by an increase in the number of phosphate-Na\textsuperscript{+} co-transport systems, and during phosphate loading (high \(P_f\) diet) the number of co-transport systems is assumed to decrease. This adaptation of the luminal transport systems by the phosphate diet is illustrated by the transport systems drawn with a broken circle. The scheme was constructed using the data of Evers \textit{et al.} (1978) and Stoll \textit{et al.} (1978).

In the following, the cellular basis of the regulation of phosphate transport in the proximal tubule by parathyrin and of the adaptation of renal phosphate transport to phosphate loading and phosphate depletion shall be discussed as a topic related to physiology. Recent studies on the effect of parathyrin injections on the transport properties of renal brush-border membranes revealed that parathyrin injection decreases specifically the capacity of the phosphate-Na\textsuperscript{+} co-transport system (Evers \textit{et al.}, 1978). The inhibition is quantitatively the same as the reduction of tubular phosphate transport \textit{in vivo}, indicating that the main, if not exclusive, regulation of tubular phosphate transport by parathyrin occurs at the luminal membrane and affects the phosphate-Na\textsuperscript{+} co-transport-system. Since injection of cyclic AMP has the same effect as parathyrin on the tubular transport and the phosphate uptake by brush-border membranes, the scheme given in Fig. 2 seems to be applicable. Parathyrin stimulates the adenylate cyclase localized exclusively at the contraluminal cell side (Shlatz \textit{et al.}, 1975) and the cyclic AMP generated inhibits the Na\textsuperscript{+}-dependent phosphate transport across the brush-border membrane.

In the adaptation of the proximal tubule to dietary phosphate uptake (decreased transport after phosphate loading and enhanced transport during phosphate depletion) other factors in addition to parathyrin seem to be involved. Stoll \textit{et al.} (1978) could show that brush-border membrane vesicles isolated from phosphate-depleted rats show a higher rate of Na\textsuperscript{+}-dependent phosphate uptake than brush-border membrane vesicles isolated from the kidneys of phosphate-loaded rats. This difference in transport is, although smaller, still observed if the imbalance in the parathyrin system, which occurs during adaption, is abolished. This indicates some further regulatory processes affecting the phosphate-Na\textsuperscript{+} co-transport system in the brush-border membrane; it is tentatively proposed in Fig. 2, that the number of transport systems...
molecules is influenced by the dietary phosphate intake, increase in number during phosphate depletion and decrease in number during phosphate loading. The effect of parathyrin on the phosphate–Na$^+$ co-transport system, on the contrary, probably involves a phosphorylation mediated by a cyclic AMP-dependent protein kinase located predominantly in the luminal membrane (Kinne et al., 1975).

Biochemical data on the molecular entity that mediates the phosphate–Na$^+$ co-transport are still limited. Kinetic studies (Hoffman et al., 1976) revealed an apparent affinity for phosphate ($K_p$) at 100mM-NaCl and pH 7.4 of 0.08mM, under the same conditions arsenate inhibits phosphate transport competitively with an inhibitor constant of 1.1mM. The transport is stimulated with increasing pH, suggesting that the phosphate ion transported preferentially by the transport system is (HPO$_4^{2-}$). When the activation of the transport system by Na$^+$ was investigated in more detail, it became evident that more than one, probably two, Na$^+$ ions are transported together with one HPO$_4^{2-}$ ion. In agreement with this assumption it was found, that the phosphate–Na$^+$ co-transport at pH 7.4 is electroneutral, i.e. independent of the electrical potential difference across the brush-border membrane. At pH 6.0, where mainly phosphate as H$_2$PO$_4^-$ is transported together with two Na$^+$ ions, the co-transport is electrogenic (Hoffman et al., 1976).

Recently isolated brush-border membranes have been used as starting material for attempts to solubilize and to purify the phosphate–Na$^+$ co-transport system. As a first step it was investigated whether it is possible to incorporate the transport system after its solubilization into liposomes in order to obtain a tool to follow the activity of the system during further purification steps (Kinne & Faust, 1977). As indicated in Fig. 3, proteoliposomes, formed by co-sonication of the high speed supernate of a Triton X-100 extract of brush-border membranes with renal phospholipids, show Na$^+$-dependent phosphate uptake which is inhibited by arsenate. Thus two main properties of the phosphate–Na$^+$ co-transport found in the intact membrane are also observed in the proteoliposomes. This indicates a successful solubilization of the transport system out of the brush-border membrane and a successful re-incorporation.
into vesicular lipid bilayers. Further purification will then make it possible to obtain information on the molecular structure of the active sites of the transport system and of the sites where regulation of the activity takes place.

Stoll, R., Kinne, R. & Murer, H. (1978) Pflügers Arch. 373, R29

Physiological Consequences of the Cellular Distribution of Sodium-plus-Potassium Ion-Dependent Adenosine Triphosphatase

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The tubular epithelium of the mammalian kidney belongs to a general class of tissues that are known collectively as transporting epithelia (Berridge & Oschman, 1972) and that are universally distributed among the coelomate metazoans. Almost exclusively, these cellular monolayers or cornified multilayers lie on the boundary between the interior of the organism and the environment. They are, however, often removed from direct contact with the exterior by systems of ducts and sphincters. As a consequence of their location, transporting epithelia are the mediators of solute exchange between the organism and its environment and are responsible for the homoeostasis of the interior. It shall be argued that these tissues carry out their diverse functions by relying upon an invariant fundamental pattern of organization. To understand the behaviour of renal tubular epithelia, these rules governing the performance of all transporting epithelia must be thoroughly grasped.

From an examination of the ultrastructure of a large number of transporting epithelia (Berridge & Oschman, 1972), it can be concluded that they are all constructed with an identical morphology (Bulger, 1965). The plasma membrane of every cell is subdivided, by the continuous ring of the tight junction, into a luminal surface in direct contact with the environment and a basolateral surface. The distribution of membrane-bound enzymes (Kyte, 1976a) and other macromolecules (Miller & Revel, 1975) displays an abrupt discontinuity at the tight junction. This observation suggests that this organelle is a boundary through which lateral diffusion cannot occur. Above and below this ring of containment, however, macromolecules appear to be distributed uniformly over the plasma membrane (Kyte, 1976b) and other macromolecules (Miller & Revel, 1975) displays an abrupt discontinuity at the tight junction. This observation suggests that this organelle is a boundary through which lateral diffusion cannot occur. Above and below this ring of containment, however, macromolecules appear to be distributed uniformly over the plasma membrane (Kyte, 1976b). The tight junction is also responsible for joining the cells together in a continuous sheet. The outermost permeability barrier thus becomes an unbroken surface composed of the luminal portions of the cell membranes, the tiles, and the tight junction, the grout. Below the tight junction lie the intercellular spaces. All of the narrow membrane-limited extracellular spaces observed within the epithelia lie between separate cells and are, as a result, directly connected with the tight junction. The contraluminal surfaces are in direct contact with a basement membrane whose permeability properties are poorly understood. A diagrammatic representation of the general morphology of transporting epithelia is presented in Fig. 1.

Without exception, it has been observed that the net flux of all solutes and solvent against their concentration gradients across transporting epithelia are eliminated by the addition of a cardiac glycoside. These ligands are highly specific inhibitors of (Na⁺+K⁺)-